## Conclusion

While ion exchange appears to be an extremely simple idea, it is used in remarkably complicated ways in living organisms. Of course, we do not know how life began but perhaps the earliest step in the direction of the development of these organized chemical systems was the formation of an ion gradient across a membrane. Once such a gradient had formed, possibly of the proton, ion exchange could be used to move some elements out of cells and others into cells. These ions could be inorganic or organic. The movements became more complicated as more membrane containments developed in evolution. Within each compartment ions could bind to form complexes or precipitates by exchange processes. We know that cells have many different kinds of ion gradient through this exchange: some relate to energy storage and some to messages by release of the gradients. Recently evolved systems are calcium triggering of muscle and sodium/potassium currents in nerves. The restoration of the gradients is very frequently energized by ion exchange across membranes. This leads us to the tantalizing problem of the movement of ions in the brain. Are ion exchange processes deeply involved in storage, memory, and in thinking? We know that the brain is an electrolytic device and hence ion movements are always active. Clearly, we could speculate at length on this topic, but what is really required is more experimental evidence concerning ion exchange in organisms and especially in the brain.

*See also:* **II/Ion Exchange:** Inorganic Ion Exchangers; Theory of Ion Exch]ange.

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# BIOLOGICALLY ACTIVE COMPOUNDS AND XENOBIOTICS: MAGNETIC AFFINITY SEPARATIONS

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## Introduction

Isolation and separation of specific molecules is used in almost all areas of biosciences and biotechnologies. Separation technology is thus one of the most important areas for further study and development. New separation techniques, capable of treating dilute solutions or solutions containing only minute amounts of target molecules in the presence of vast amounts of accompanying compounds in both small and large-scale processes, even in the presence of particulate matter, are necessary.

In the area of biosciences, isolation of biologically active compounds and xenobiotics is usually performed using a variety of chromatography procedures, affinity chromatography being one of the most important. Affinity ligand techniques currently represent the most powerful tool available for downstream processing both in terms of their selectivity and recovery. The strength of column affinity chromatography has been shown in thousands of successful applications, especially on a laboratory

scale. The disadvantage of standard column procedures is the impossibility of such systems to cope with samples containing particulate material so they are not suitable for use in the early stages of the isolation/purification process where suspended solid and fouling components are present. In this case magnetic affinity batch adsorption, applications of magnetically stabilized fluidized beds or magnetically modified two-phase systems have shown their usefulness.

The basic principle of magnetic affinity separation is very simple. Magnetic carriers bearing an immobilized affinity ligand or magnetic biopolymer particles are mixed with a sample containing target compound(s). Samples may be crude cells lysates, whole blood, plasma, urine, cultivation media, water, soil extracts and many others. Following an incubation period when the target compound(s) bind to the magnetic affinity particles, the whole magnetic complex is easily and rapidly removed from the sample using an appropriate magnetic separator. After washing, the isolated target compound(s) can be eluted and used for further work.

Magnetic separation techniques have several advantages in comparison with standard separation procedures. The separation process can be performed directly in crude samples containing suspended solid material. Due to the magnetic properties of the magnetic affinity particles (and the diamagnetic properties of the majority of the contaminating molecules and particles present in the treated sample) they can be relatively easily and selectively removed from the sample. In fact, magnetic separation is the only feasible method for recovery of small magnetic particles (diameter ca. 0.1–1 μm) in the presence of biological debris and other fouling material of similar size. Moreover, the power and efficiency of magnetic separation procedures is especially useful for large-scale operations. The magnetic separation techniques are also the basis of various automated procedures, especially magnetic particle-based immunoassay systems for the determination of a variety of analytes. The basic equipment for laboratory experiments is very simple. Magnetic particles of various types are easily available, as well as magnetic separators. A short description is given below.

## Equipment and Materials

Magnetic carriers with immobilized affinity ligand or magnetic particles prepared from a biopolymer exhibiting affinity for the target compound(s) are used to perform the isolation procedure. Magnetic separators are necessary to recover magnetic particles from the system.

*Magnetic carriers and adsorbents* are commercially available and can also be prepared in the laboratory. Such materials are usually available in the form of magnetic particles prepared from various synthetic polymers, biopolymers or porous glass, or magnetic particles based on inorganic magnetic materials such as surface-modified magnetite can be used. In fact, many of the particles behave like paramagnetic or superparamagnetic ones responding to an external magnetic field, but not interacting themselves in the absence of a magnetic field. This is important due to the fact that magnetic particles can be easily resuspended and remain in suspension for a long time. The diameter of the particles is from ca. 50 nm to ca. 10 μm. Magnetic particles having a diameter larger than ca. 1 μm can be easily separated using simple magnetic separators, while separation of smaller particles (magnetic colloids with a particle size ranging between tens and hundreds of nanometers) may require the use of high-gradient magnetic separators.

Commercially available magnetic particles can be obtained from a variety of companies. In most cases polystyrene is used as a matrix, but carriers based on cellulose, agarose, silica, porous glass or silanized magnetic particles are also available. Particles with immobilized affinity ligands are available, oligodeoxythymidine, streptavidin, antibodies, protein A and protein G being used most often. Magnetic particles with such immobilized ligands can serve as generic solid phases to which native or modified affinity ligands can be immobilized (e.g. antibodies in the case of immobilized protein A, protein G or secondary antibodies, biotinylated molecules in the case of immobilized streptavidin or adenylated molecules in the case of immobilized oligodeoxythymidine). In exceptional cases, enzyme activity may decrease as a result of usage of magnetic particles with exposed iron oxides. In this case encapsulated microspheres, having an outer layer of pure polymer, are safer. In **Table 1** is given a list of companies producing and selling magnetic particles of various types.

In the laboratory, magnetite (or similar magnetic materials such as maghemite or ferrites) particles are usually surface modified by silanization. This process modifies the surface of the inorganic particles so that appropriate function groups become available, which enable easy immobilization of affinity ligands.

Biopolymers such as agarose, chitosan, κ-carrageenan and alginate can be easily prepared in a magnetic form. In the simplest way, the biopolymer solution is mixed with magnetic particles and after bulk gel formation the magnetic gel formed is broken into fine particles. Alternatively, biopolymer solution containing dispersed magnetite is dropped into a mixed hardening solution or a water-in-oil suspension

**Table 1** Examples of commerically available magnetic particles suitable for magnetic affinity separations

| *Manufacturer/supplier* | *Name of particles* | *Diameter ($\mu$m)* | *Polymer composition/surface modification* | *End groups/ activation possibility* | *Immobilized ligands* |
|---|---|---|---|---|---|
| Advanced Biotechnologies, Epsom, UK | XM200 Microsphere | 3.5 | Polystyrene | –COOH | Oligo (dT), antibodies, streptavidin, protein A |
| | Magnacil | 0.5–3.5 | Silica | Silica | |
| Bangs Labs, Fishers, IN, USA | Magnetic Microspheres | ~ 1 | Styrene–divinyl benzene copolymer | –COOH, $-NH_2$ | Streptavidin, protein A, antibodies |
| Cortex Biochem, San Leandro, CA, USA | MagaCell | | Cellulose | –OH | Streptavidin, protein A, protein G, oligo (dT), DEAE, CM, PEI |
| CPG, Lincoln Park, NJ, USA | MPG | 5 | Porous glass | –SiOH, glyceryl, $-NH_2$, hydrazide | Streptavidin, avidin |
| Dynal, Oslo, Norway | Dynabeads M-280 | 2.8 | Polystyrene | Tosyl activated | Streptavidin, oligo (dT), antibodies |
| | Dynabeads M-450 | 4.5 | | | |
| | Dynabeads M-500 | 5.0 | | | |
| Immunotech, Marseille, France | Iobeads | ~ 1 | | | Antibodies, avidin |
| Merck, Darmstadt, Germany | Biobeads | 1 | Polystyrene | | Streptavidin |
| | | 1–3 | Silica | | |
| Novagen, USA | Magnetight | | | | Oligo (dT) |
| PerSeptive Biosystems, Farmingham, MA, USA | BioMag | 0.5–1.5 | Silanized iron oxides | –COOH, $-NH_2$ | Antibodies, protein A, protein G, streptavidin, biotin |
| Prolabo, Fontenay-Sous-Bois, France | Estapor | ~ 1 | Polystyrene | –COOH, –OH, $-NH_2$ | |
| Promega, Madison, WI, USA | MagneSphere Paramagnetic Particles | | | | Streptavidin |
| ProZyme, San Leandro, CA, USA | Magnetic beads | 1.4 | Styrene–divinyl-benzene copolymer | | Streptavidin, protein A, protein A/G, protein G |
| | | 2.2 | | | |
| | | 2.3 | | | |
| Qiagen | Ni-NTA Magnetic agarose beads | 20–70 | Agarose | | Nitrilotriacetic acid |
| Quantum Magnetics, USA | Magnetic particles | 0.05 | | | Streptavidin, DEAE, CM, C18, protein A, silica |
| | | 0.25 | | | |
| Scigen, Sittingbourne, UK | M 100 | 1–10 | Cellulose | –OH | Streptavidin, biotin, oligo (dT) |
| | M 104 | | | | |
| | M 108 | | | | |
| | Magnetic agarose | 1–5 | Agarose | | |
| Seradyn, Indianopolis, IN, USA | Sera-Mag | 1 | | –COOH | Streptavidin, oligo (dT) |
| Spherotech, Libertyville, IL, USA | SPHERO magnetic particles | 1–4.5 | Polystyrene | –COOH, $-NH_2$ | Streptavidin, biotin, antibodies |

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technique is used to prepare spherical particles. Basically the same procedures can be used to prepare magnetic particles from synthetic polymers such as polyacrylamide or poly(vinylalcohol).

In one of the approaches used, standard affinity chromatography material is post-magnetized by pumping the water-based ferrofluid through the column packed with the sorbent. Magnetic material accumulates within the affinity adsorbent pores thus modifying the chromatography material into magnetic form.

Some affinity ligands (usually general binding ligands) are already immobilized to commercially available carriers (see **Table 1**). To immobilize other ligands to both commercial and laboratory-made magnetic particles, standard procedures used in affinity chromatography can be employed. Usually functional groups available on the surface of magnetic particles such as –COOH, –OH or $-NH_2$ are used for immobilization; in some cases, magnetic particles are already available in the activated form (e.g. tosyl activated).

*Magnetic separators* are necessary to separate the magnetic particles from the system. In the simplest approach, a small permanent magnet can be used, but various magnetic separators employing strong

rare-earth magnets can be obtained at reasonable prices. Commercial laboratory scale *batch magnetic separators* are usually made from magnets embedded in disinfectant-proof material. The racks are constructed for separations in Eppendorf microtubes, standard test tubes or centrifugation cuvettes. Some have a removable magnetic plate to facilitate washing of separated magnetic particles (**Figure 1**). Other types of separators enable separations from the wells of microtitration plates and the flat magnetic separators are useful for separation from larger volumes of suspensions (up to ca. 500–1000 mL).

*Flow-through magnetic separators* are usually more expensive and more complicated, and *high-gradient magnetic separators* (*HGMS*) are typical examples (**Figure 2**). Laboratory-scale HGMS are constructed from a column packed with fine magnetic-grade stainless-steel wool or small steel balls placed between the poles of an appropriate magnet. The suspension is pumped through the column, and magnetic particles are retained within the matrix. After removing the column from the magnetic field, the particles are retrieved by flow and usually by gentle vibration of the column.

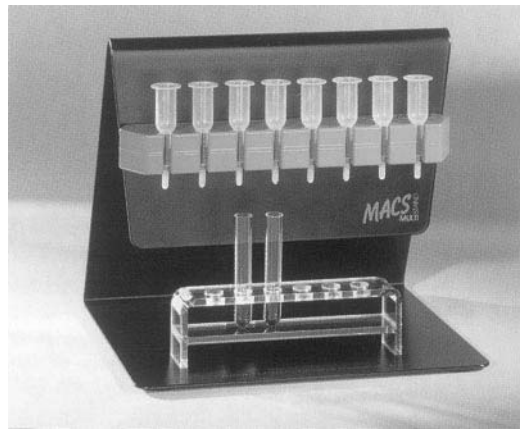


**Figure 2** (See Colour Plate 62). A typical example of laboratory-scale high-gradient magnetic separators. OctoMACS Separator (Miltenyi Biotec, Germany) can be used for simultaneous isolation of mRNA. Courtesy of Miltenyi Biotec, Germany.

## Basic Principles of Magnetic Affinity Separations

In general, magnetic affinity separations can be performed in two different modes. In the *direct method* an appropriate affinity ligand is directly coupled to the magnetic particles or biopolymer exhibiting affinity towards target compound(s) is used in the course of preparation of magnetic affinity particles. These particles are added to the sample and target compounds then bind to them. In the *indirect method* the free affinity ligand (in most cases an appropriate antibody) is added to the solution or suspension to enable the interaction with the target compound. The resulting complex is then captured by appropriate magnetic particles. In case antibodies are used as free affinity ligands, magnetic particles with immobilized secondary antibodies, protein A or protein G are used for capturing the complex. Alternatively, the free affinity ligands can be biotinylated and magnetic particles with immobilized streptavidin or avidin are used to capture the complexes formed. In both methods magnetic particles with isolated target compound(s) are magnetically separated and then a series of washing steps are performed to remove the majority of contaminating compounds and particles. The target compound is then usually eluted, but for specific applications (especially in molecular biology, bioanalytical chemistry or environmental chemistry) they can be used still attached to the particles, such as in the case of polymerase chain reaction, magnetic ELISA, etc.

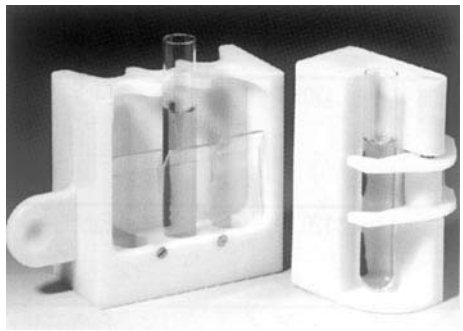

**Figure 1** (See Colour Plate 61). Examples of test-tube magnetic separators (Dynal, Norway). Left, Dynal MPC-6; right, Dynal MPC-1. Courtesy of Dynal, Oslo, Norway.

The two methods perform equally well, but, in general, the direct technique is more easily controlled. The indirect procedure may perform better if affinity ligands have poor affinity for the target compound.

In some cases, nonspecific binding of accompanying compounds can be observed due to the specific properties of the magnetic particle material. In this case, pretreatment with the magnetic carrier without immobilized affinity ligand or with immobilized nonspecific molecules will usually help. The nonspecific binding can be also minimized by adding a nonionic detergent both in the sample and in the washing buffers after isolation of the target.

In most cases, *magnetic batch affinity adsorption* is used to perform the separation step. This approach represents the simplest procedure available, enabling the whole separation to be performed in one test-tube or flask. If larger magnetic particles (with diameters

ca. > 1 μm) are used, simple magnetic separators can be employed. If magnetic colloids (diameters ranging between tens and hundreds of nanometers) are used as affinity adsorbents, high-gradient magnetic separators have usually to be used to remove the magnetic particles from the system.

Alternatively, *magnetically stabilized fluidized beds* (MSFB), which allow continuous separation, can be used. The use of MSFB is an alternative to conventional column operation, such as packed bed or fluidized bed, especially for large-scale purification of biological products. Magnetic stabilization enables the expansion of a packed bed without mixing of solid particles. High column efficiency, low pressure drop and elimination of clogging can be attained.

Biocompatible two-phase systems, composed for example from dextran and polyethylene glycol, are often used for isolation of biologically active compounds, subcellular organelles and cells. The separation of the phases can be accelerated by the addition of fine magnetic particles or ferrofluids to the system followed by the application of a magnetic field. Magnetically enhanced phase separation usually increases the speed of phase separation by a factor of about 10 in easy systems, but it may increase by a factor of many thousands in difficult systems.

## Examples of Magnetic Affinity Separations of Biologically Active Compounds and Xenobiotics

Magnetic affinity separations have been successfully used in various areas, such as molecular biology, biochemistry, immunochemistry, enzymology, analytical chemistry and environmental chemistry. **Tables 2–4** show some selected applications of these techniques.

At present, magnetic affinity separation techniques are used especially in molecular biology for the isolation of RNA, DNA and oligonucleotides. Almost all the procedures employ the same basic principle, based on the hybridization of immobilized oligonucleotides and target structures. There are several companies offering oligodeoxythymidine immobilized on magnetic particles, which can be effectively used for rapid isolation of highly purified, intact poly $A^+$ mRNA from eukaryotic total RNA. Poly $A^+$ mRNA has been successfully isolated from various samples, such as cells, animal and plant tissues, blood, cells isolated by immunomagnetic separation, paraffin-embedded tissues, etc. Also cells and tissues containing high RNase activities can be used for mRNA isolation. The separated mRNA can be eluted from the beads by lowering the ionic strength of the elution buffer and used for further applications (Northern blotting, dot–blot hybridization, hybridization probes) or used still bound to magnetic particles (cDNA synthesis, construction of solid-phase cDNA library, etc.). Enzymatic downstream applications are usually not inhibited by the presence of magnetic particles. The covalent binding of oligodeoxythymidine to magnetic particles makes it possible to regenerate the specific adsorbent and to reuse it up to four times.

Isolation of DNA and RNA can be simply performed using biotinylated specific nucleic acids or oligonucleotides immobilized on magnetic particles with immobilized streptavidin. Usually large binding capacity can be achieved resulting in excellent reaction kinetics and high efficiency of the procedure. In addition, magnetic silica particles have been used for simple isolation of DNA and RNA from various biological samples and also to purify DNA fragments after agarose gel electrophoresis.

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**Table 2** Typical examples of magnetic separations of nucleic acids

| *Nucleic acid* | *Magnetic system used* | *Typical examples* |
|---|---|---|
| RNA | Magnetic particles with immobilized oligo $(dT)_{25}$ | Eukaryotic poly $A^+$ mRNA, viral poly $A^+$ RNA |
| | Magnetic particles with immobilized specific oligonucleotides | tRNA |
| DNA | Dynabeads DNA DIRECT | PCR-ready DNA |
| | Biotinylated cloned genomic DNA immobilized on Dynabeads Streptavidin | cDNA |
| | Dynabeads M-280 Streptavidin with immobilized biotinylated oligonucleotide complementary to the lacZ region | M13 single-stranded DNA |
| | Magnetic particles with immobilized pyrimidine oligonucleotide | Double-stranded target DNA (triple helix formation) |
| | COOH-terminated magnetic beads (under specific concentrations of polyethylene glycol and salt) | Double-stranded DNA, PCR products, M13 single-stranded DNA |
| | Magnetic silica particles | DNA |

**Table 3** Selected examples of magnetic affinity separation of proteins

| *Isolated protein* | *Magnetic system used* | *Typical examples* |
|---|---|---|
| Enzymes | Sub-micron ferrite particles with immobilized soybean trypsin inhibitor | Trypsin |
| | Ferrofluid-modified 5′-AMP-Sepharose 4B | Alcohol dehydrogenase |
| | Magnetic agarose beads with immobilized dye | Lactate dehydrogenase |
| | Magnetic chitin | Lysozyme |
| | Dynabeads with immobilized polyclonal antibodies | Angiotensin-converting enzyme |
| | Iminodiacetic acid coupled to magnetic agarose and charged with $Zn^{2+}$ | AngioI-TEM-$\beta$-lactamase |
| | Alginate-magnetite beads | Pectinase |
| | Magnetic affinity aqueous two-phase system | Hexokinase |
| Antibodies | Magnetic particles with immobilized antibodies against human IgG | Human IgG antibodies |
| | Magnetic particles with immobilized protein A or protein G | Antibodies |
| | Human serum albumin immobilized onto ferromagnetic dacron | Antibodies against human serum albumin |
| Lectins | Magnetic cross-linked chitosan | *Solanum tuberosum* lectin |
| Receptors | Dynabeads M-450 sheep anti-mouse $IgG_1$ with immobilized monoclonal antibody | Human transferrin receptor |
| | Magnetic particles with immobilized oligonucleotide containing EcdR binding sequence | Ecdysteroid receptor (EcdR) from *Drosophila melanogaster* |
| Other proteins | Magnetic particles with immobilized DNA/RNA fragment containing the specific binding sequence | DNA/RNA binding proteins |
| | Magnetic particles with immobilized *m*-aminophenylboronic acid | Glycated haemoglobin |
| | Organomercurial-agarose magnetic beads | Transcriptionally active chromatin restriction fragments with accessible histone H3 thiols |
| | Ni-NTA Magnetic agarose beads | 6xHis-tagged proteins |

**Table 4** Selected examples of magnetic separation of low-molecular-weight biologically active compounds and organic and inorganic xenobiotics

| *Type of compound* | *Magnetic system used* | *Typical examples* |
|---|---|---|
| Biologically active compounds | Magnetic particles with immobilized aldosterone antiserum | Aldosterone |
| | Molecularly imprinted polymer containing magnetic iron oxide | (S)-propranolol |
| | Magnetic charcoal | Separation of free antigens in radioimmunoassays |
| Organic xenobiotics | Magnetic particles with immobilized Cu-phthalocyanine | Polyaromatic hydrocarbons, triphenylmethane dyes |
| | Magnetic polyethyleneimine microcapsules | Carcinogens |
| | Magnetic particles with immobilized specific antibodies | Pesticides, polyaromatic hydrocarbons, TNT, PCBs |
| | Bacterial cells adsorbed to magnetite | Chlorinated hydrocarbons and pesticides |
| | Magnetic charcoal | Water-soluble dyes, pesticides |
| Inorganic xenobiotics | Magnetic chitosan | $Cu^{2+}$ ions |
| | Cells of *Enterobacter* spp. immobilized on magnetite | $Ni^{2+}$ ions |
| | Magnetic cross-linked cell walls of *Saccharomyces cerevisiae* | Ions of heavy metals |
| | Magnetotactic bacteria | Ions of heavy metals |
| | Solvent extractants on magnetic microparticles | Radionuclides |

In the case of protein separation no simple strategy for magnetic affinity separations exists. Various affinity ligands have been immobilized on magnetic particles, or magnetic particles have been prepared from biopolymers exhibiting affinity for target enzymes or lectins, as shown in **Table 3**. Immunomagnetic particles, i.e. magnetic particles with immobilized specific antibodies against the target structures, have been used for the isolation of various antigens, both molecules and cells and can thus be used for the separation of specific proteins. Enzyme isolation is usually performed using immobilized inhibitors, cofactors, dyes or other suitable ligands, or magnetic beads prepared from affinity biopolymers are used. A general procedure, especially from the point of view of recombinant oligohistidine-tagged proteins, is based on the application of metal chelate magnetic adsorbents. Another general procedure employs immobilized protein A or protein G for the specific separation of immunoglobulins from ascites, serum and tissue culture supernatants.

Magnetic separation of low-molecular-weight biologically active compounds has been used in the course of their determination by various types of immunoassays. Usually immunomagnetic particles directly capture the target analyte, or magnetic particles with immobilized streptavidin are used to capture the complex of biotinylated primary antibody and the analyte. The separated analyte is then determined using an appropriate method.

Isolation and separation of organic and inorganic xenobiotics from environmental and clinical samples using magnetic techniques may find useful applications in the near future. Immobilized copper phthalocyanine dye has an affinity for planar organic compounds, such as polyaromatic hydrocarbons with three and more fused aromatic rings in their molecules, and for triphenylmethane dyes, both groups representing real or potential carcinogens and mutagens. Immunomagnetic separation of xenobiotics such as pesticides, TNT or PCBs is used as a first step in the course of their immunoassay.

Magnetic solid-phase extraction (MSPE) enables preconcentration of target analytes (e.g. environmental contaminants) from large volumes of solutions or suspensions using relatively small amount of magnetic specific adsorbent. This procedure can substitute the standard liquid–liquid and solid-phase extraction procedures.

## Future Perspectives

The isolation and separation of biologically active compounds and xenobiotics using magnetic affinity techniques are a relatively new approach and still under development. Due to the commercial availability of magnetic affinity particles and kits these techniques are currently used mainly in molecular biology (especially for separation of nucleic acids) and as parts of the kits for the determination of selected analytes using magnetic ELISA and related techniques (especially determination of clinical markers and environmental contaminants). Up to now small-scale separations prevail and thus the full potential of these techniques has not been fully exploited.

It can be expected that further development will be focused on two areas. The first one will be oriented to the laboratory-scale application of magnetic affinity separation techniques in biochemistry and related areas (isolation of a variety of both low- and high-molecular weight substances of various origins directly from crude samples thus reducing the number of purification steps) and in biochemical and environmental analysis (application of immunomagnetic particles for separation of target analytes from a mixture followed by their detection using ELISA and related principles). Such a type of analysis enables portable assay systems to be constructed for the detection and determination of environmental contaminants directly on site or for near-patient analysis of various disease markers. Alternatively, fully automated systems for the detection of clinical markers will be constructed.

In the second area, larger-scale (industrial) systems will be developed and used for the isolation of biologically active compounds directly from the crude culture media, wastes from food industry, etc. It is not expected that large amounts of low-cost products will be isolated using magnetic techniques, but attention will be directed to the isolation of minor, but highly valuable components present in raw materials. Of course, prices of magnetic carriers have to be lowered, and special types of low-cost, biotechnology-applicable magnetic carriers prepared by simple and cheap procedures have to become available. Magnetic separations could thus be the technique for the 21st century.

**See Colour Plates 61, 62.**

*See also:* **I/Affinity Separation. II/Affinity Separation:** Immunoaffinity Chromatography. **Extraction:** Solid-Phase Extraction; Solvent Based Separation. **III/Catalyst Studies: Chromatography:** Isolation Magnetic Techniques; DNA. **Immunoaffinity Extraction: RNA. Appendix 1/Essential Guides for Isolation/Purification of Cells: Essential Guides for Isolation/Purification of Enzymes and Proteins: Essential Guides for Isolation/Purification of Nucleic Acids. Appendix 2/Essential Guides to Method Development in Affinity Chromatography.**

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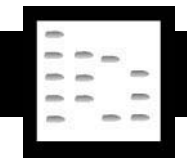
# BIOMEDICAL APPLICATIONS



## Gas Chromatography – Mass Spectrometry

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### Introduction

The tremendous technological developments that have followed the initial interfacing of gas chromatographs with mass spectrometers together with the phenomenal advances in computerized data handling have provided an analytical technique that finds practically universal application. The biomedical sciences afford an enormous range of applications of this instrumentation where its full potential as a primary method for the separations and identification of extremely complex mixtures is clearly demonstrated.

The applications can be grouped into three broad categories based upon the nature of the analytes:

- Small volatile molecules, e.g. metabolites, xenobiotics (drugs, toxins, etc), food components
- Large labile molecular species, e.g. biomacromolecules and even whole cells
- Isotopomers (molecules differing only in isotopic composition), e.g. tracer studies, isotopic labelling, breath gas diagnostics, natural abundance studies

The analysis of small volatile molecules, perhaps after derivatization, is the major application area; particular biomedical applications in clinical chemistry and occupational hygiene are illustrated below. Others are exemplified under the headings sport, environment, food and forensics. The analysis of biomacromolecules and whole cells is another rapidly expanding field but using other mass spectrometric and separatory techniques (electrospray, atmospheric pressure and matrix assisted laser desorption ionization; see also LC–MS, CE–MS). Gas chromatography–mass spectrometry (GC–MS) of pyrolysates can provide information about otherwise intractible materials.

The elucidation of mechanisms and biochemical pathways using tracer and labelling techniques is an example of the third category of applications which is also a rapidly expanding area with the wider availability of stable as opposed to radioactive labelled compounds. The ability to separate and distinguish between components in a complex mixture that differ solely in their isotopic composition allows exogenous materials to be distinguished from endogenous species; it also provides a means of improving quantitation (via isotope dilution) and allows dynamic studies of metabolism or dysfunction to be undertaken. It is this latter area of GC–SIRMS (GC–stable isotope ratio MS) that will be emphasized.

### Analysis of Small Volatile Molecules

#### Instrumentation

The majority of instruments utilize capillary columns thereby allowing relatively simple connection to the mass spectrometer. Earlier systems used packed columns that required some form of separator in order